Rennet-Induced Milk Coagulation by Continuous Steady Shear Stress

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The effect of continuous steady shear stress (CSSS) on rennet-induced coagulation of milk was studied by measuring the change in viscosity of the system with time. Continuous shear stress (≤ 0.5 Pa) applied during coagulation did not counteract the network formation at standard cheese-making conditions. In fact, CSSS of 0.2 Pa promoted coagulation by possibly increasing diffusion, collision, and hydrolization rates due to near-field, attractive hydrodynamic reactions. This was evidenced by the high viscosity of the resultant coagulum. However, the viscosity profile of the coagulum formed in the presence of CSSS followed the same trend as that formed in the absence of CSSS. Viscosity versus time profiles in both cases displayed an initial lag phase followed by a steady increase until a plateau value. The viscosity plateau reached under CSSS was marked with several sudden peaks, indicating the dynamic structure of the coagulum. These peaks in viscosity profiles began to appear after about 1380 s since rennet addition at standard cheese-making conditions. The time at which viscosity first exceeds 40 kPa s was verified to coincide with manual determination of the time at which the coagulum is cut (i.e., cutting time) during cheese making. © 2002 Elsevier Science (USA)

Key Words: rennet; milk; coagulation; shear stress; cutting time; cutting time peak.

INTRODUCTION

Cheese manufacturing begins with coagulation. This step is initiated by the cleavage of milk proteins using rennet, a proteolytic enzyme complex (chymosin–pepsin). The milk-clotting enzymes attack the κ-casein in milk and cleave the Phe_{105}-Met_{106} bond. As a result, hydrophilic glycomacropeptide is released into solution and hydrophobic p-κ-casein is left on the micelle. This reaction is referred to as the primary phase of coagulation. When a sufficient proportion of hydrolyzed κ-casein has been generated, the lower net negative charge of the micelle and the greater hydrophobicity of p-κ-casein lead to aggregation (secondary phase). This time for incipient aggregation, known as the "clotting time", can be determined by the Berridge test (1). As coagulation proceeds, fat globules, water, and water-soluble materials are trapped in the aggregating protein matrix, first forming linear chains and later branched structures (2). When the branched structure, or continuous curd, reaches a certain degree of firmness, it is severed into small pieces to initiate syneresis. Determination of this time, referred to as the "cutting time", is crucial in cheese making. If the curd is cut too soon, most of the fat globules, and some curd fines, are lost into the serum, decreasing the yield. If the curd is cut too late, the coagulum has mostly stabilized and there will be limited micelle reassociation, so the product will retain more water, decreasing the shelf life of the final product. Currently, the cutting time is determined mainly by empirical evaluation or at a set time after rennet addition. Although there have been several attempts to define the cutting time objectively, a clear definition and an objective measure are still lacking.

A colloidal suspension, such as the coagulating milk, can be either in a dispersed or in a flocculated state depending on such factors as the magnitude of particle–particle interaction energy and the particle concentration. Flocculation of colloidal suspensions may be initiated by varying the interparticle interaction energy. Varying the reaction conditions such as pH or adding surfactants and/or applying hydrodynamic forces will inherently cause the transition from the dispersed to the flocculated state. The aggregation of particles is determined by the frequency of particle collisions. Three types of particle collision mechanisms are known to exist: perikinetic aggregation, differential sedimentation, and orthokinetic aggregation. Perikinetic aggregation is caused by random movement of particles due to Brownian motion. Differential sedimentation occurs when particles of varied sizes settle at different rates. Hydrodynamically induced particle collisions are the basis for orthokinetic aggregation.

Applying shear forces to solutions will align and orient the molecules in the direction of shear flow, causing an increase in molecular interactions and, thus, inducing structural changes. Several researchers have observed the formation of anisotropic structures in binary mixtures of liquids (3), polymer systems (4), and surfactant solutions (5). It has also been demonstrated that many wormlike micellar solutions exhibit shear-thickening behavior under the presence of an applied shear field (6, 7). Oda

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[2] Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

et al. (8) studied the influence of shear flow on the structure of wormlike micelles. They used rheological, electrical conductivity, and optical birefringence measurements to study Gemini surfactant 12-2-12 in solutions of heavy water, with concentrations ranging from 0.05 to 2.5%. They reported shear-thickening behavior (onset shear thickening) in concentrations up to 1.8% with a characteristic critical shear rate. Before the critical shear rate, viscosity of the solution was independent of shear rate. At the critical shear rate, the viscosity increased until it reached a maximum (shear thickening), but then started to decrease (shear thinning). A critical shear rate was also observed in the electrical-conductivity experiments, supporting the results of rheological experiments. With use of optical birefringence experiments, the samples were found to be isotropic at low shear rates and anisotropic at high shear rates. The time to equilibrium decreased as the shear rate increased. They also examined cryo transmission electron micrographs of the 0.8% in the frozen hydrated state. At rest, the micrographs showed a homogenous dispersion of micelles in various sizes (9). Under applied shear, the micrographs displayed mainly micelles with a network-like structure. These pictures provided direct evidence for shear-induced aggregation of the micelles. Finally, the researchers concluded that, under shear, micelles in solution first form isotropic aggregates. Increasing the rate of shear increases the size of aggregates. As the size of aggregates approaches the size of the couette gap, a strong anisotropy is created. After cessation of the applied shear, the anisotropy is destroyed. The aggregates then disintegrate, reverting back to a homogenous solution with wormlike micelles.

Mendes *et al.* (10) reported a shear-induced transition from a vesicle-like to wormlike micelles in the presence of the surfactant cetyltrimethyl ammonium 3-hydroxy naphthalene-2-carboxylate (CTAHNC). They used a step-shear method at 35 and 60°C. After shear rates of 0.1, 2, 9, and 16 s^{-1} were applied for 20, 10, 10, and 10 min, respectively, the stress was recorded at 2-s intervals. Optical micrographs taken at 60°C indicated wormlike micelles in the system. At 35°C, the team reported a predominance of vesicles at low shear rates; however, at high shear rates, the recorded stresses at 35 and 60°C were similar, indicating the formation of micelle structures induced solely by shear. To support these conclusions, they conducted small-angle neutron scattering (SANS) experiments. The plots of scattering vector versus scattering intensity showed a very sharp peak when the shear rate increased; a second peak developed after 59 s^{-1}. The appearance of these peaks were attributed to wormlike micelles. Structural changes were also induced by shear. The SANS results demonstrated shear-induced conversion of vesicles to micelles. These shear-induced conversions were reported to be identical to those induced by high temperature and the addition of surfactants such as cetyl trimethylammonium bromide (CTAB), Triton X-100, and sodium 3-hydroxynaphthalene-2-carboxylate (SHNC).

It is well known that the addition of organic counterions to cationic surfactant solutions initiates micellar growth. Hofmann *et al.* (11) studied the rheological and birefringence behavior on drag-reducing surfactant solutions of tallow-(tris-hydroxy ethyl)-ammonium acetate (ETHOQUAD T/13-50)/sodium salicylate (NaSal) mixtures. The addition of NaSal reduced the drag when shear-induced supramicellar structures (SIS) were formed. The process appears to begin with the formation of aggregates. Then, long arrays, resembling strings of pearls, orient themselves in the direction of shear. As the salt/surfactant molar ratio (x) increased above 1.5, they observed the development of shear moduli along with SIS at small shear rates. At higher salt concentrations shear moduli increased. Shear-induced behavior was most pronounced at $x = 2.5$. At certain concentrations, partially ordered, network-like micellar patterns and fully aligned SIS micelles coexisted. Beyond this regime no SIS was observed. They theorized that SIS only exists when there is a stearically hindered formation, which is not available in the dense structures of network-like micelles. Micelles first develop into aggregates and then align themselves in the direction of shear, and finally the network starts to form. NaSal plays a major role in these structural changes by covering the positive surface charges and, thus, fostering attractive hydrophobic interactions.

Smoluchowski (12) derived collision-frequency equations for perikinetic and orthokinetic coagulation; however, Smoluchowski's model took only one component of the relative velocity gradient into account. By contrast, Camp and Stein (13) introduced multiple components of the relative velocity gradient, although their model lacked the directional nature of particle collisions. Kramer and Clark (14) evaluated the components of the strain-rate tensor on coagulation kinetics. They found that the collision frequency is a function of both shear and normal components of the strain-rate tensor. Thus, they obtained a scalar quantity called "the absolute maximum principal strain rate, $|a'_{max}|$" from the strain-rate tensor by diagonalization to its principal normal components. Following this new model, the total mass transferred in hydrodynamically driven particle collisions along with the collision frequency function is used for more accurate calculations of orthokinetic aggregation.

$$|a'_{max}| = \left(\frac{2\omega R_o^2 R_i^2}{(R_o^2 - R_i^2)^2} \right) \ln \frac{R_o}{R_i}. \qquad [1]$$

In this scalar quantity, R_o and R_i are the outside and inside radius of the couette apparatus, respectively, and ω is the frequency. This equation is valid for a local subregion of fluid under the influence of linear velocity gradients. It takes into account the mass transfer of particles and can be applied to a wide range of particle sizes but it ignores near field interactions.

Axford (15) used photon correlation spectroscopy (PCS) to obtain cluster size distributions of orthokinetically aggregating latex spheres of diameters 264 and 303 nm. The results showed a monodisperse distribution of the spheres initially. Then, the average size of the clusters increased, leading to the first peak. At the second peak, fewer clusters appeared. Eventually, the second peak held bigger clusters and the primary peak disappeared. This second peak became self-preserving and symmetrical.

OBJECTIVES

The specific objectives of this investigation were to

(1) determine the viscosity vs time profile during rennet-induced coagulation of milk under CSSS;

(2) compare the viscosity profiles obtained under CSSS to those obtained under intermittent shearing;

(3) determine if any features of the viscosity profile can be correlated with subjective coagulum cutting time and determine the effect of cheese-making variables such as pH, temperature, $CaCl_2$, and fat, protein, and rennet concentration on the viscosity profile under CSSS;

(4) validate cutting time determination utilizing the cutting time peak of the viscosity profile of rennet-induced coagula under CSSS during cheese making.

MATERIALS AND METHODS

Sample Preparation

Grade A, low-heat, spray-processed, pasteurized, non-fat-dry-milk (NFDM) powder (Dairy America, Dublin, CA), rennet, $CaCl_2$, acetic acid, and Dean's brand dairy cream (36% fat) were purchased from the Dairy Plant of the Food Science Department at the University of Wisconsin—Madison. Rennet is MAXIREN (100% Chymosin) from *Kluyveromyces lactis* with NaCl, propanediol, and sodium benzoate. Its suggested usage is 28.35–56.70 g/454 kg of milk with a 1/40 dilution. Cream and rennet were kept at the refrigerator temperature ($5 \pm 2^\circ$C). Milk powder, $CaCl_2$ (31–35%), and acetic acid were kept at ambient temperature ($25 \pm 3^\circ$C).

Milk was prepared by dissolving the NFDM powder in distilled water at 32°C for 2 h and stored in a refrigerator overnight for complete hydration. On the day of the experiment, after the temperature was adjusted, cream was added by manual mixing for 2 min. Then, $CaCl_2$ was added and allowed to equilibrate for 5 min. The pH was adjusted as desired by adding acetic acid. After 15 min, rennet was introduced, marking the start of coagulation.

Experiments

There were six experimental parameters each at three treatment levels: (a) enzyme concentration (30, 35, and 45 mL/454 kg of milk), (b) temperature (30, 32, and 34°C), (c) pH (6.2, 6.35, and 6.5), (d) $CaCl_2$ concentration (0, 90, and 180 mL/454 kg of milk), (d) protein (3, 3.5, and 4%), and (e) fat (0, 2, and 3.6%).

These values were calculated considering that NFDM consists of 3.16% water, 36.16% total protein, 0.77% fat, 51.98% lactose, and 7.98% ash (standard values) and cream consists of 36% fat, 58.88% water, 1.95% total protein, 2.75% lactose, and 0.42% ash (Dean's brand dairy cream). The range of experimental variables covered the possible ranges during cheesemaking and seasonal variations in milk.

There were two sets of experiments:

(1) Viscosity measurement:
   (a) of rennet-induced milk coagulation under CSSS;
   (b) of rennet-induced milk coagulation under intermittent shear stress;
   (c) of milk.
(2) Clotting time (CLT) and manual cutting time (CT) determinations.

Viscosity Measurements

Rheological properties of rennet-induced milk coagulation were measured using a controlled-stress dynamic rheometer (Bohlin CVO, Bohlin Instruments Inc., Cranbury, NJ) with a double-gap couette geometry (DG). The DG consists of an outer cylinder with an inverted hollow cylinder (outer diameter 45.46 mm, inner diameter 43.80 mm), which is lowered into an annulus (outer diameter 50 mm, inner diameter 39.82 mm), creating two gaps for the sample. The outer gap is 4.54-mm thick and the inner gap is 3.98-mm thick. The immersion depth is 46 mm. It has a large surface area, making it ideal for low-viscosity, low shear rate tests. The shear stress as well as the shear rate vary slightly over the gap.

For viscosity profile measurement during coagulation, a 30- mL milk sample was used. Casein micelles, which constitute 80–82% of the milk proteins, are on the order of 20–600 nm. The micelles are several orders of magnitude smaller than the gaps in the DG. Different stress levels were used in the preliminary tests before selecting 0.2 Pa for CSSS and intermittent shearing measurements. Sampling frequency for both measurements is 29 s and the fraction of flow time required for measurement is 1.1 s. There was no delay time (no flow history) applied for intermittent shearing measurements. Thus, for intermittent shearing measurements the coagula was at a quiescent state during the 29-s sampling period. The viscosity of milk was measured by stress ramp tests (0.002–0.2 Pa).

Clotting Time and Manual Cutting Time Determinations

For the clotting time determinations, several test tubes with 10 mL of milk were kept inclined at a 30° angle in a water bath set at the experimental temperatures. The test tubes were rotated at 2 rpm in a rotating spindle. The water bath was illuminated by a 100-W electric lamp to enable easy observation of the aggregate formation. The time elapsed from the addition of rennet to the visual observation of the incipient aggregate forming on the wall of the test tubes was recorded as the CLT.

For manual CT determination (the current industry practice) milk was coagulated in a milk vat at the Dairy Plant of Food Science Department at the University of Wisconsin—Madison. The cut time was determined by an experienced cheesemaker inserting a knife into coagulum. Time at which the coagulum separates easily and the whey looks clean as the knife is lifted was recorded as the cut time (16).

RESULTS AND DISCUSSION

The rennet-induced coagulation in the presence of CSSS was analyzed in terms of the prevailing shear rates at different constant shear stresses (Fig. 1). For this, we compared the viscosities of the primary phase (lag phase) and the secondary phase (plateau value). Strikingly, at shear stresses of 5 and 10 mPa we did not record a lag phase. The viscosity at these shear stresses started increasing immediately upon rennet addition, indicating a threshold shear stress where the fraction of collisions that are successful are higher initially even in the presence of stearic hindrances. This indicates that applied hydrodynamic forces may induce coagulation of milk (higher attractive potential) even before any hydrolization takes place. However, the plateau viscosity of these coagula was also lower, indicating incomplete hydrolization. Beyond this threshold value, colliding micelles repulsed each other rather than forming doublets due to increased shear rates (increased collision frequency with decreased capture efficiency). The plateau viscosity of coagulum was higher at higher applied shear stresses, indicating decreased capture efficiency initially, but perhaps with increased hydrolization rates due to increased collision frequency. The shortest (time scale) and the smallest lag phase viscosity recorded was at a shear stress of 0.2 Pa (among 0.05, 0.1, and 0.2 Pa) with the highest plateau viscosity.

The CSSS experiments conducted on rennet-induced milk coagulation at various shear stress levels indicated that the maximum CSSS for orthokinetic aggregation at standard cheesemaking conditions was 0.5 Pa. An applied shear stress level of 0.2 Pa was observed to yield optimal results for cutting time determinations. Thus, an applied shear stress of 0.2 Pa was used for the rest of the experiments.

Viscosity Profile of Rennet-Induced Coagulation of Milk under CSSS

The linear plots (Fig. 2) of viscosity vs time for coagulating milk under 0.2 Pa of CSSS exhibited a steady baseline, marked with several peaks. These peaks were observed to occur prior to the empirically determined cutting time. However, the lag phase was not distinguishable. When the same data were plotted on a semi-logarithmic scale (Fig. 2), the lag phase (660 s) was clearly observable. After the lag phase, the viscosity increased very rapidly. After a while, approximately 1200 s, the viscosity of milk coagula approaches a plateau value, which was similar for other experimental parameters (17). This is closely followed by the appearance of abrupt peaks (around 1380 s).

The peaks in the viscosity versus time plots may occur for several reasons. The initial rise of the peak may correspond to the formation of numerous long chains or strands especially as they assemble in line. Then, these strands and small aggregates begin to interact with each other to form a branched structure. The subsequent decrease in viscosity takes place as the particles aggregate, forming a loose network. These effects are possibly exaggerated by the applied shear. In addition, peaks continue
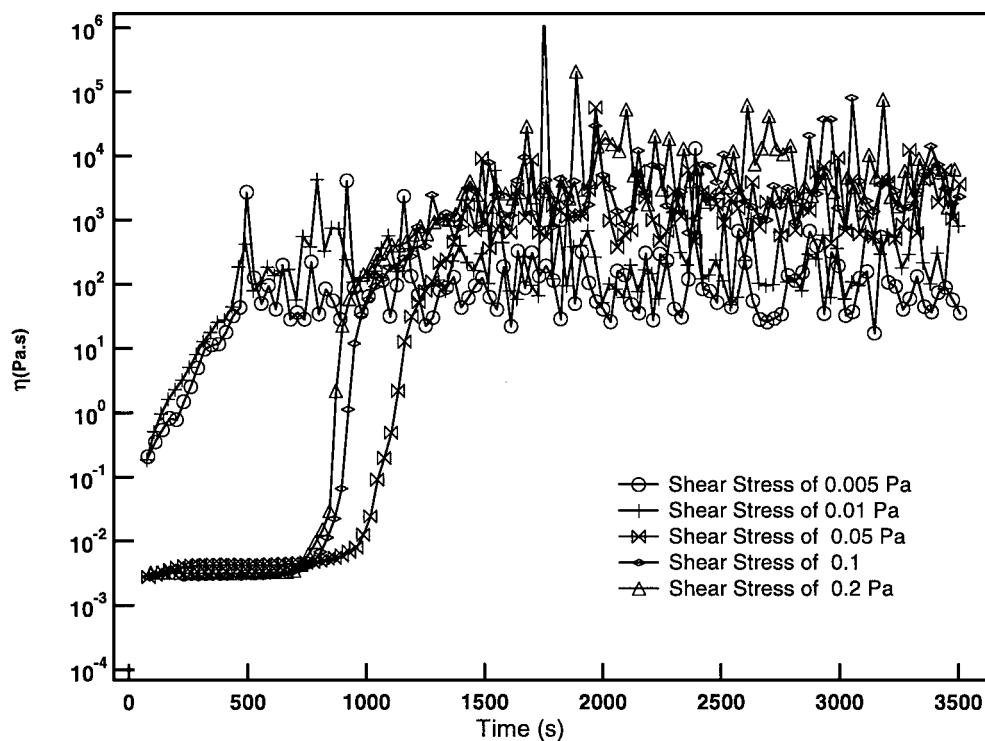

FIG. 1. Viscosity of rennet-induced milk coagulation at 32°C and standard cheese-making conditions of varying CSSS levels.
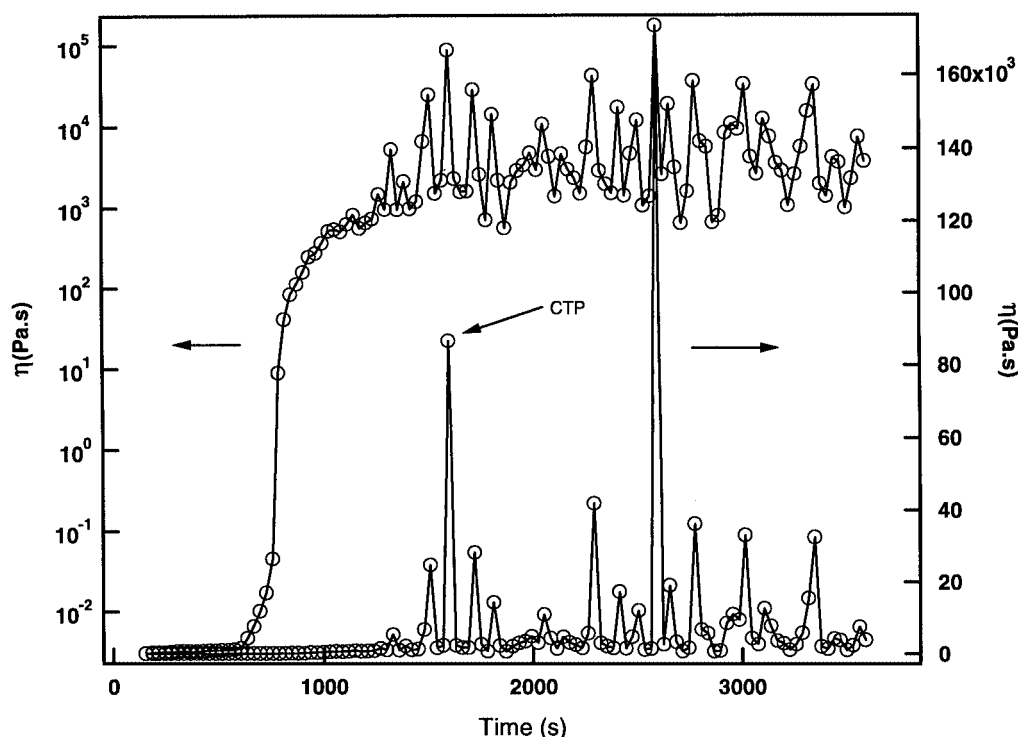
FIG. 2. Viscosity of rennet-induced milk coagulation in a linear and a semi-log scale at standard cheese-making conditions by CSSS of 0.2 Pa at 32°C.

to be observed after the CT because the gel is still forming. During this time some of the bonds that are already formed are strengthened, while some of them break and re-form (18). We think that as the strands separate from the network, the viscosity increases (upslope of the peak); as these chains get reconnected back to the network, the viscosity decreases (downslope of the peak).

Comparing Viscosity Profiles Obtained under CSSS and Intermittent Shearing

The trends of the viscosity and corresponding shear rate profiles (Figs. 3a and 3c) of rennet-induced milk coagulation under CSSS are similar to those of rennet-induced milk coagulation under intermittent shearing, except for fluctuations in the plateau region. Both plots show an initial lag phase, followed by a plateau. However, the viscosity values of the lag phases and the plateau values differ greatly, indicating orthokinetic aggregation. Green *et al.* (19) examined the lag phase during rennet-induced milk coagulation and stated that the measured viscosity will be experiment dependent. Although their results, obtained with a rotational viscometer, indicated only a small decrease in viscosity of the coagulating milk system during the lag phase, the measurements with an Ostwald viscometer (20) showed a 80–85% decrease from milk's viscosity. Kopelman and Kogan (21) supported these findings. Similarly, Lomholt and Qvist (22) measured the viscosity of the primary phase using a Viscotek viscometer. They found that the viscosity of the lag phase de-

creased 3% (it is believed that these measurements are reliable within ±5%) with the max shear rate being 310 1/s. Our results show the lag phase viscosity under intermittent shearing to be 0.1 Pa s and under CSSS to be 3 mPa s with the corresponding shear rates of 2 and 66 1/s, respectively. These are approximately 45 and 1.36 times higher than that of the milk viscosity, respectively. The high viscosity of the lag phase by intermittent shearing may partly be due to the non-Newtonian behavior of milk (Fig. 3b) at low shear rates. The non-Newtonian viscosity of milk at low shear rates has also been observed by other researchers (23). In addition, the low shear stress (0.2 Pa) applied intermittently prevail on zero hydrodynamic forces on the rennet-induced milk coagulation, reflecting more credible changes in the system. However, the viscosity measured under intermittent shear stress corresponding to a shear rate of 20–30 1/s would yield truer coagulation viscosity. In addition, the slight difference of viscosity found by CSSS measurements might be due to the introduced Taylor vortices, calculated and observed, for milk using DG of 43 1/s. However, both results indicate that the viscosity of the lag phase is not less than the milk viscosity, which supports the findings of Green *et al.* (19). Furthermore, contrary to Lomholt and Qvist (22), steady shear stress of 0.5 Pa applied during coagulation (corresponds to a shear rate of 100 1/s and more) inhibited the formation of milk coagulum.

The viscosity plateau region of the coagulum measured using CSSS is marked by several abrupt peaks. This is expected because the ongoing cross-linking of the protein strands, along with fusion of particles, will lead to stresses in the network.
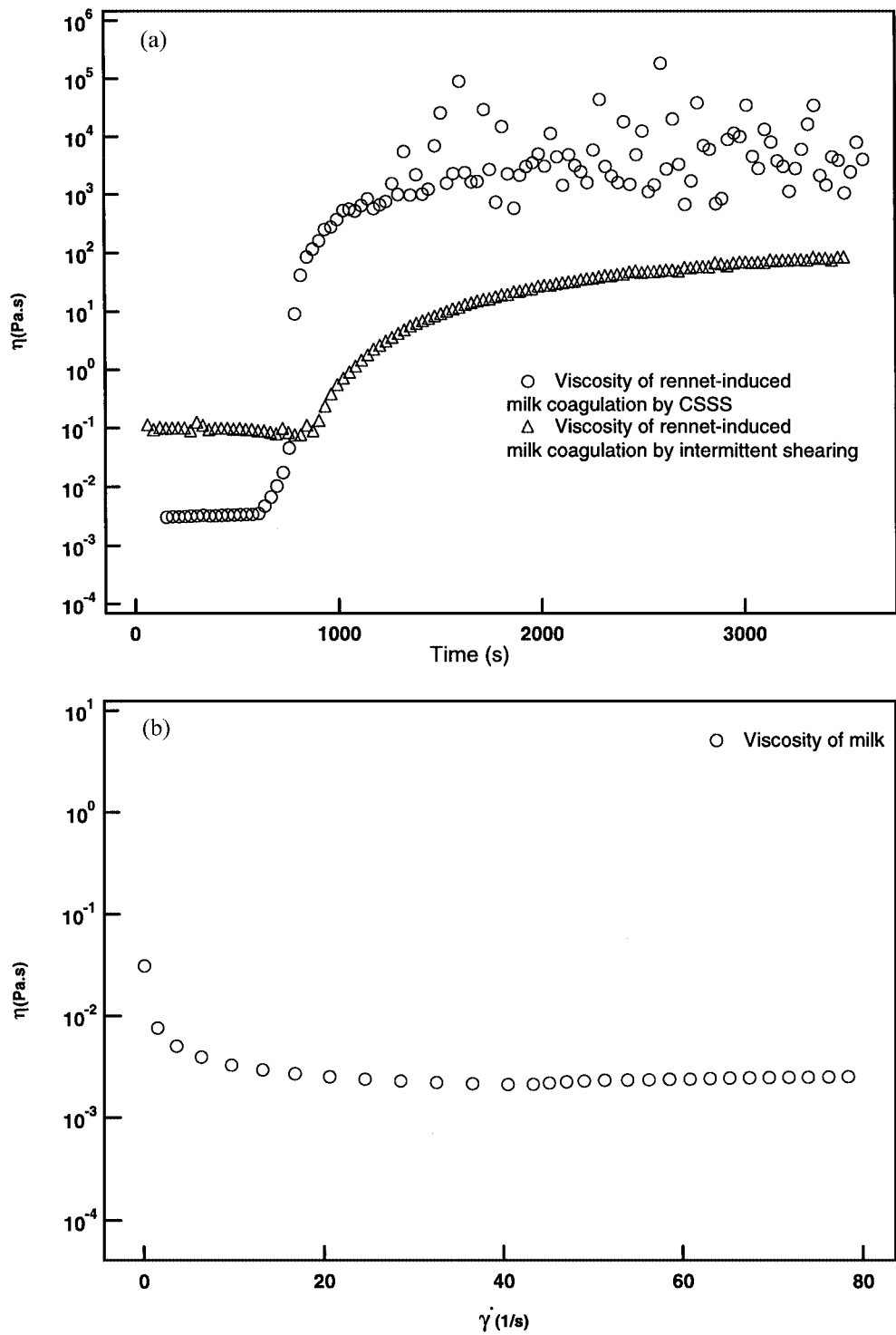
FIG. 3. Viscosities of rennet-induced milk coagulation (a) and milk (b) and corresponding shear rates (c) measured by intermittent and CSSS shearing (0.2 Pa) at 32°C and standard cheese-making conditions.

Thus, bonds between the structural elements will relax and re-form. These rearrangements at a molecular and subparticle level continue even after the coagulum is formed. Continuous shearing during coagulation does have additional consequences. At about 2520 s the viscosity reaches 2 kPa s, which is an over 40-fold increase over the corresponding value obtained under intermittent shearing. This probably reflects increased collision rates, diffusion rates, and capture efficiency, along with the
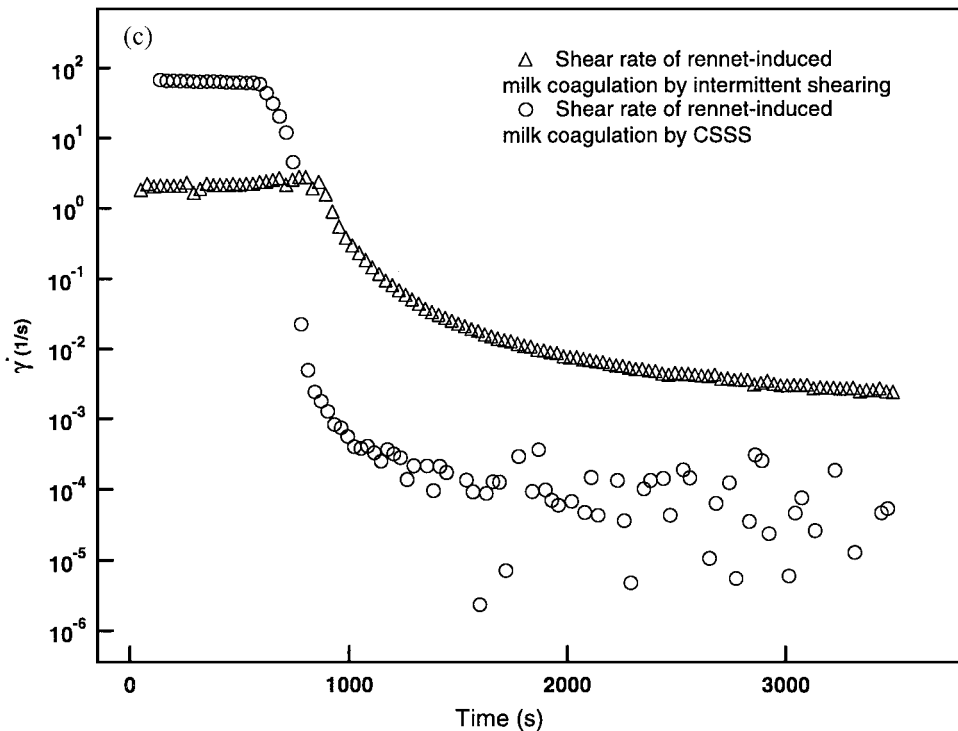
FIG. 3—*Continued*

micelles aligned in the direction of shear and increased interactions between casein micelles. In addition, there may be increased hydrolization due to increased diffusion rates of rennet. Colloidal casein micelles are sterically stabilized by κ-casein molecules, which consists of polyelectrolyte-type protein, called glycomacropeptide. As the casein micelles progressively lose their steric stability, they became very adhesive perhaps because of the exposure of the nonpolar amino acid side chains on the surface of the molecule (2). Then, they may participate in a variety of interactions (e.g., hydrophobic, hydrogen bonding, ion bonding) and thus aggregate extensively due to increased functional sites. Overall, differences found between the two coagula (under CSSS and intermittent shearing) are thought to be due to the main interaction forces between casein molecules.

Cutting Time Determination Utilizing the Viscosity Profile of Rennet-Induced Coagula under CSSS and Effect of Cheese-Making Variables on the Viscosity Profile

The time at which the first significantly large peak was recorded coincided with the manually determined cut time (1680 s). This peak is noted as the cutting time peak (CTP) on Fig. 2. Green *et al.* (24) examined the intermicellar relationships in rennet-treated milk to study the process of gel assembly. They analyzed the coagulation and firming process with seven electron micrographs taken over a range between 0 and 300% of the CLT. At 200% CLT they observed a loose network of strands, created by a chain of micelles grouping together. At 300% CLT,

the strands were 5 micelles thick, spaced apart by a width of about 10 micelles. The micelle chains appeared to form bridges between the micelles. Our CTP, the first peak in the viscosity plots which is greater than 40 kPa s, which was validated as the CT determined empirically in the Dairy Plant of the Food Science Department at the University of Wisconsin—Madison, was observed on an average at about 254% CLT at standard cheese-making conditions. Thus, we speculate that the formation of CTP coincides with the formation of a loose network.

The effects of experimental parameters on CT are summarized in Table 1 and analyzed by ANOVA. ANOVA, single-factor analysis of variance, helps to determine whether samples are statistically different by calculating the F-test statistic. The computed F statistic utilized the p value (the probability value) at 5% significance level to determine whether to reject the null hypothesis. If a p value of ≤ 0.05 is obtained, the treatments in the ANOVA are considered to be significantly different.

Changing the protein amount, in the limits used, did not have a significant effect on the CT ($p > 0.05$). The CT of low-fat coagulum was longer; however, the CT for skim milk only decreased slightly ($p > 0.05$). When the maximum Ca level allowed in cheesemaking was used, the CT decreased significantly ($p < 0.05$). A lack of calcium addition, on the other hand, did not have an effect on the CT ($p > 0.05$). Increasing the rennet level decreased the CT significantly ($p < 0.05$), and decreasing it did not significantly change the CT ($p > 0.05$). This was contrary to findings of small-amplitude oscillatory-shear experiments (17). The plateau storage modulus for increased rennet

TABLE 1
Results of Rennet-Induced Milk Coagulation by CSSS of 0.2 Pa Shear Stress at 32°C

Experiment ID*	Average CLT (s) ±s.d.	Average CT (s) ±s.d.	Average %CLT at CT ±s.d.
Standard cheese making condition	708 ± 45	1704 ± 170^a	246 ± 35
Same as standard w/4% total protein	885 ± 60	1778 ± 200^a	203 ± 19
Same as standard w/3% total protein	757 ± 35	1917 ± 231^a	247 ± 50
Same as standard w/2% fat	903 ± 66	1900 ± 312^a	212 ± 20
Same as standard w/no added fat	848 ± 31	1742 ± 354^a	208 ± 37
Same as standard at pH 6.35	471 ± 17	1028 ± 62^b	224 ± 11
Same as standard at pH 6.2	347 ± 6	717 ± 161^b	219 ± 52
Same as standard w/30 mL Rennet	804 ± 38	1592 ± 128^a	202 ± 8
Same as standard w/45 mL Rennet	591 ± 80	1292 ± 88^b	225 ± 17
Same as standard w/180 mL CaCl$_2$ per 454 kg of milk	625 ± 106	1083 ± 29^b	181 ± 27
Same as standard w/no added CaCl$_2$	811 ± 47	1708 ± 123^a	214 ± 19
Same as standard at 34°C	735 ± 27	1358 ± 166^b	188 ± 21
Same as standard at 30°C	892 ± 38	1817 ± 126^a	205 ± 10

Note. Standard cheese making conditions are 3.5% total protein, 3.6% fat, 32°C, pH 6.5, and 35 ml rennet and 90 ml CaCl$_2$ per 454 kg of milk. Data identified with superscript "a" are not significantly different from the standard cheese making condition, and those with superscript "b" represents a significant difference from the standard cheese making condition by ANOVA.

concentrations was not different than the standard cheesemaking conditions and for decreased rennet concentrations it was found to be significantly lower. Increasing the temperature by 2°C decreased the CT significantly ($p < 0.05$). In contrast, decreasing the temperature by 2°C increased the CT slightly (larger average, $p > 0.05$). Interestingly, plateau storage modulus for higher temperature was not statistically different than the standard cheese-making conditions and for the lower temperature it was significantly lower (17). Using a pH of 6.35 greatly shortened the CT. Decreasing pH even further to 6.2 decreased the CT most dramatically.

The average CLT is 708 s at standard cheese-making conditions. This time is recorded right after the viscosity starts to increase. Although the CLT closely resembles the gel point determined by SAOS experiments (17), our results do not exhibit the weight-average molecular weight diverging to infinity. In analogy, the experimental results of Green and Morant (25) suggest a sigmoidal relationship between the weight-average degree of polymerization and the time of aggregation process. In addition, they calculated the number of functional sites to be equal to 1.8 until 300% CLT, even though both linear chains and small aggregates were present. This might mean that at the gel point of rennet-induced milk coagulation there are still many individual casein micelles as well as indefinitely large structures. Their results were also supported by Hyslop and Qvist (26), who applied different kinetic models to describe rennet-induced coagulation of casein micelles. The best model was the one with an aggregation rate constant depending on the number of free sites on the particles; however, the calculations indi-

cated the number of functional sites at the end of proteolysis to be slightly less than two, which is the lower limit for linear aggregation.

As can be seen from Table 1, the clotting time (CLT) for the coagulum prepared with high-protein milk, skim milk, low rennet, and low temperature were all long, but interestingly the %CLT at the CT were short. These results indicated that although the primary phase of the rennet-induced milk coagulation under CSSS was delayed by these factors, the secondary phase was completed earlier. Furthermore, factors like high calcium and very low pH affected the primary phase significantly (decreased CLT) and the recorded CTs were much shorter. Interestingly, the %CLT at CT was only slightly smaller for high-calcium conditions with not much change observed for lowered pHs. As the amount of protein increases in the milk, the increased collisions between micelles accelerates the intermicellar linkages, even though it might delay the primary phase. The earlier gel formation in skim milk may occur because there are no fat globules to interrupt the collision of casein micelles, thus increasing the capture efficiency and alignment of the micelles. The low rennet concentrations and low temperatures also caused lower %CLT levels at the CT with increased CLT. Interestingly, the maximum calcium added in the rennet-induced milk coagulation by CSSS affected the aggregation mechanism most profoundly. As a result, the secondary phase was completed sooner with a significantly shortened CT. Perhaps in this case the negative charges of the micelles were shielded, causing faster coagulation and/or also divalent ions may have cross-linked between the micelles. Higher temperatures also shortened the %CLT at CT. Researchers have long known that the temperature has the most pronounced effect on the flocculation reaction (secondary phase) due to the increased fusion of the micelles. Finally, decreasing the pH had a tremendous effect on the primary phase. The lower the pH, the faster the rate of enzymatic phase with a maximum rate at pH 6.0. Lowering the pH also affects aggregation because it allows for a faster fusion of micelles due to higher electrostatic attraction. All of these CTs determined by CTPs are in accordance with the expected CTs determined empirically.

Validation of Cutting Time Determination Utilizing the CTP of the Viscosity Profile of Rennet-Induced Coagula under CSSS during Cheesemaking

The CTs determined by the CSSS method were also validated three times in the Dairy Plant of the Food Science Department, University of Wisconsin (Madison, WI). The varieties of cheeses (27) made were 50% reduced fat Cheddar (Fig. 4a), Swiss (Fig. 4b), and Gouda (Fig. 4c). We compared the CT results estimated by the CSSS method with those estimated by the Dairy Plant cheesemaker. The CTP for Gouda cheese occurred at 21 min and the viscosity was 80 kPa s. The cheesemaker estimated the CT to be 20 min. The Swiss cheese was cut by the cheesemaker at 25 min after adding the rennet. The CTP of about 40 kPa s was observed at 25 min after rennet addition. For the
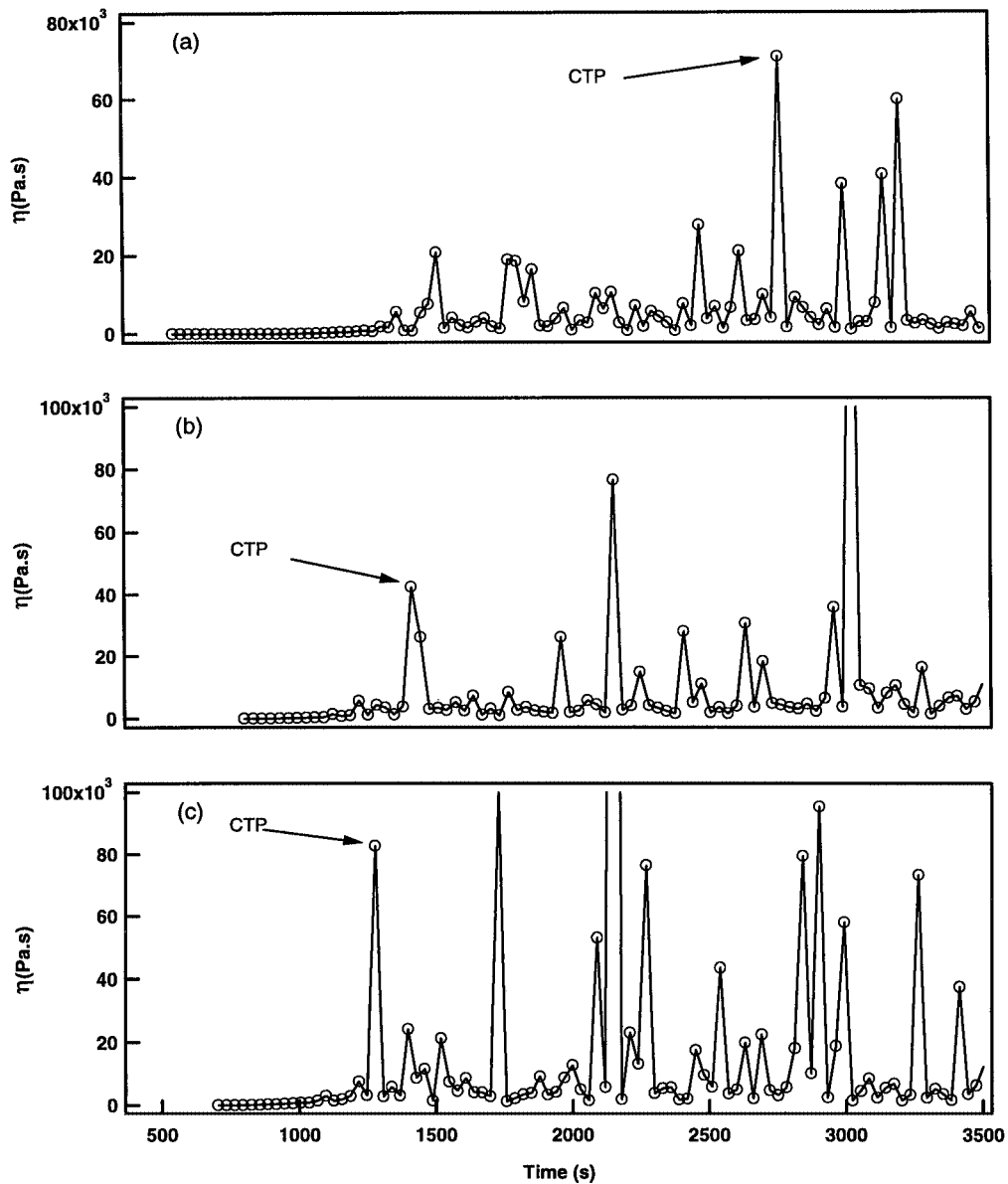
FIG. 4. Viscosity of rennet-induced milk coagulation during (a) 50% reduced fat Cheddar making at 32°C, (b) Swiss cheese making at 32°C, (c) Gouda cheese making at 30°C, at the Dairy Plant using CSSS of 0.2 Pa.

last experiment one vat of 50% reduced fat Cheddar cheese was made and the cheese was cut according to the CTP criterion. The cheesemaker's recommended CT was 2 min before the CSSS method (45 min by CSSS). The CTP viscosity for this cheese was approximately 70 kPa s. Typically, the firmness at cutting would be greatest for reduced fat Cheddar, softest for Swiss, and intermediate for Gouda cheese (28). These trends were also exhibited by the CSSS method considering that perhaps CTP viscosity as well as the CT contributes to the firmness of the gel. According to the cheesemaker, the result of the cheese composition tests (17) for 50% reduced fat Cheddar cut according to the CTP criterion had a slightly higher fat-in-dry base than it should have,

indicating an increased yield using the CT indicated by the CSSS method.

CONCLUSIONS

Our research shows that continuous steady shear stresses lower than 0.5 Pa favor the rennet-induced coagulation of milk. Shearing at low-stress levels actually accentuates the coagulation process rather than interrupting the formation of the gel. This may be due to increased diffusion rates, collision rates, and capture efficiency (hydrodynamic forces acting on colloidal casein). In addition, applied shear stress may increase the proteolysis

rates. Also, under applied shear stress, the molecules will be aligned in a more parallel fashion, which offers less resistance to coagulation. As a result, rennet-induced milk coagulum by CSSS was 40 times more viscous in the plateau region than intermittent shearing. Although the mechanism of aggregation and interacting forces may differ in rennet-induced milk coagulation by CSSS, the phases of aggregation nonetheless mimic those seen in coagulation induced by rennet alone at 0.2 Pa of shear stress level. Furthermore, the measured viscosities of cheese-making parameters and the Dairy Plant trials indicated that the empirically determined cutting time seems to correspond to the viscosity of at least 40 kPa s.

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